AD_____

Award Number:
W81XWH-09-1-0720

TITLE:
"Impact of Obesity on Tamoxifen Chemoprevention in a Model of Ductal Carcinoma in Situ"

PRINCIPAL INVESTIGATOR:
Sarah Dunlap-Smith, Ph.D.

CONTRACTING ORGANIZATION:
University of Texas at Austin
Austin, Texas   78712

REPORT DATE:
October 2010

TYPE OF REPORT:
Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick, Maryland   21702-5012

DISTRIBUTION STATEMENT:

    x Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-10-2010	Annual Summary	15 Sep 2009 - 14 Sep 2010

4. TITLE AND SUBTITLE

Impact of Obesity on Tamoxifen Chemoprevention in a Model of Ductal Carcinoma in Situ.

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0720

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sarah Dunlap-Smith, Ph.D.

uctcj 0uo kj B o ckr0wgzcu0gf w

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas at Austin   Austin, Texas 78712

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research   Fort Detrick, Maryland
And Materiel Command   21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
No abstract provided.

15. SUBJECT TERMS
Obesity, Calorie Restriction, Breast Cancer, Mouse Model

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU	15	**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION: Obesity increases risk for breast cancer in postmenopausal women and increases mortality in pre- and postmenopausal women, in fact, 30-50% of breast cancer deaths in post-menopausal women may be attributed to excess body weight (*1*). The HER-2/neu proto-oncogene is amplified in 25-30% of human primary breast cancers, and increased levels of HER-2/neu expression in tumors can have a negative impact on prognosis of the cancer (*2*). Approximately two-thirds of breast cancers arising in postmenopausal women are positive for estrogen receptor (ER) in carcinoma cells. However, tumors negative for estrogen receptor (ER-) confer a much worse prognosis (*3*). Energy balance modulation through diet-induced obesity and calorie restriction has been shown to modulate serum levels of many growth factors and hormones, including estrogen. Additionally, energy balance modulation affects cancer initiation and progression multiple mouse and primate models (*4*). However, the specific mechanisms by which obesity affects ER- breast cancer risk or prognosis are not clearly understood, and strategies for offsetting the negative effects of obesity are urgently needed, so the purpose of my project is twofold. First, we will determine which obesity-related growth factors/hormones are key to tumor progression. Second, we will determine how blocking specific growth factors can decrease the negative effects of obesity on breast cancer and increase response to chemopreventive drugs (chemopreventive drugs are compounds given to *prevent* breast cancer, i.e. tamoxifen). This annual report summarizes the characterization of the effects of dietary energy balance modulation on metabolic hormones and mammary tumor development, growth, and progression in MMTV-erbB2 (HER-2/neu overexpressing) mice (**Specific Aim 1**).

BODY: In our original proposal, we hypothesized that: ***Obesity-induced increases in circulating IGF-1 levels promote IGF-1/ER crosstalk in the mammary epithelium, leading to a reduction in the chemopreventive efficacy of tamoxifen.*** In Specific Aim 1, we outlined the **characterization the effects of dietary energy balance modulation on metabolic hormones and mammary tumor development, growth, and progression in MMTV-erbB2 mice.** The model in which we have chosen to test this hypothesis is the MMTV-erbB2 transgenic mouse model, in which mice were fed a standard diet-induced obesity regimen. **Task 1** was to modulate diets in MMTV-erbB2 mice and measure effects on tumor. The first **Milestone** was to receive IACUC approval, which was accomplished on 13 August 2008, renewed on 06 July 2009, and on 03 August 2010, and as needed for personnel and minor modifications. The USAMRMC has been notified of all major and minor protocol modifications. **Task 1.a** was accomplished when 45 MMTV-erbB2 were mice ordered and put on diet regimens. Blood samples were taken for fasting glucose measurements and for serum hormone analysis (**Task 1.b**). As tumors became palpable in the transgenic mice, tumor weight measurements were taken (**Task 1.c**). Tissues were harvested from the mice at the time of sacrifice, including tumor, mammary fat pad, liver, visceral white adipose tissue, skin, and skeletal muscle (**Task 1.d**). We also assessed body composition, food consumption, and time to tumor appearance in the MMTVerbB2 mice.

We found that the high fat diet regimen significantly increased body weight and percent body fat through increased caloric intake (HF vs. Control; p<0.0001). The 30% caloric restriction significantly decreased body weight and percent body fat through decreased caloric intake (CR vs. Control; p<0.0001) (**Figure 1**). Importantly, we found that after 60 weeks of feeding a high-fat diet-induced obesity regimen (60% kcal from fat), a control ad-libitum diet (10% kcal from fat), or a 30% calorie restriction regimen (reduction calculated versus Control caloric intake; isonutrient), CR significantly increased survival

of MMTV-erbB2 mice (p=0.01) (**Figure 2**). In a subset of mice, which were sacrificed at 8 weeks-old, we found that MMTV-erbB2 mice begin to lose ERα expression very early in life. A significant difference in expression can be detected in mammary fat pad (MFP) between 6 week and 8 week-old mice (p=0.03). In 8 week-old MMTV-erbB2 mice, a trend exists linking CR to increased ERα expression and HF to decreased ERα expression. We also found that CR significantly increases ERβ expression. In fact, CR increases ERβ expression to levels even higher than those at the Baseline timepoint, and was significantly increased from Control diet animals (p<0.0001) (**Figure 3**). ERβ expression is thought to have a protective effect from breast cancer (*5, 6*), and could be a putative mechanism for CR-mediated cancer prevention in this model.

KEY RESEARCH ACCOMPLISHMENTS:

- High-fat, Control, and Calorie-Restriction diet regimens caused significant differences in body weights, caloric intake, and body composition of MMTV-erbB2 mice.
- Calorie restriction significantly increases lifespan in MMTV-erbB2.
- MMTV-erbB2 began to lose ERα expression in mammary fat pad very early in life; a significant difference was detected between 6 week and 8 week-old mice.
- After 2 weeks of diet, CR significantly increased ERβ expression and a trend showed HF diet decreased ERβ expression.

REPORTABLE OUTCOMES:

No reportable outcomes have yet resulted from this research.

CONCLUSION:

We have accomplished all tasks outlined in the original proposal for the first year of the study. We found that calorie restriction is a potent inhibitor of MMTV-erbB2 tumor initiation, likely through decreased circulating IGF-1 mediation of ER expression in the mammary fat pad. We are currently analyzing the full serum hormone panel of these mice, and elucidating the differences in signaling pathways downstream of the IGF-1 receptor and the estrogen receptor. We are currently on schedule to complete Specific Aim 2 in Year 2 of funding, and will use this model to analyze the mechanism of obesity-driven tamoxifen resistance. Specific Aim 3 is scheduled to be completed in Year 3. Currently, no changes will be made to the study design of Specific Aims 2 and 3.

This study will lay the foundation for larger translational/clinical studies investigating the role of IGF-1 in promoting the adverse effects of obesity on breast tumor development and progression, and validating suppression of IGF-1 pharmacologically as an effective chemopreventive approach. A significant gap in the literature exists in elucidating the impact of circulating obesity-related hormones on the chemopreventive response to tamoxifen. The successful accomplishment of our proposed aims could have a significant impact on the development of prevention strategies for obesity-related breast cancer in high-risk women.

REFERENCES:

1. E. E. Calle, C. Rodriguez, K. Walker-Thurmond, M. J. Thun, *The New England journal of medicine* **348**, 1625 (Apr 24, 2003).
2. D. J. Slamon *et al.*, *Science (New York, N.Y* **244**, 707 (May 12, 1989).
3. A. C. Wolff *et al.*, *Arch Pathol Lab Med* **131**, 18 (2007).
4. S. D. Hursting, S. M. Smith, L. M. Lashinger, A. E. Harvey, S. N. Perkins, *Carcinogenesis* **31**, 83 (Jan, 2010).
5. A. Tsezou *et al.*, *Breast* **17**, 159 (Apr, 2008).
6. G. Lazennec, D. Bresson, A. Lucas, C. Chauveau, F. Vignon, *Endocrinology* **142**, 4120 (Sep, 2001).
7. B. Cardinali, L. Fiore, N. Campioni, A. De Dominicis, P. Pierandrei-Amaldi, *J Virol* **73**, 7070 (1999).

SUPPORTING DATA:
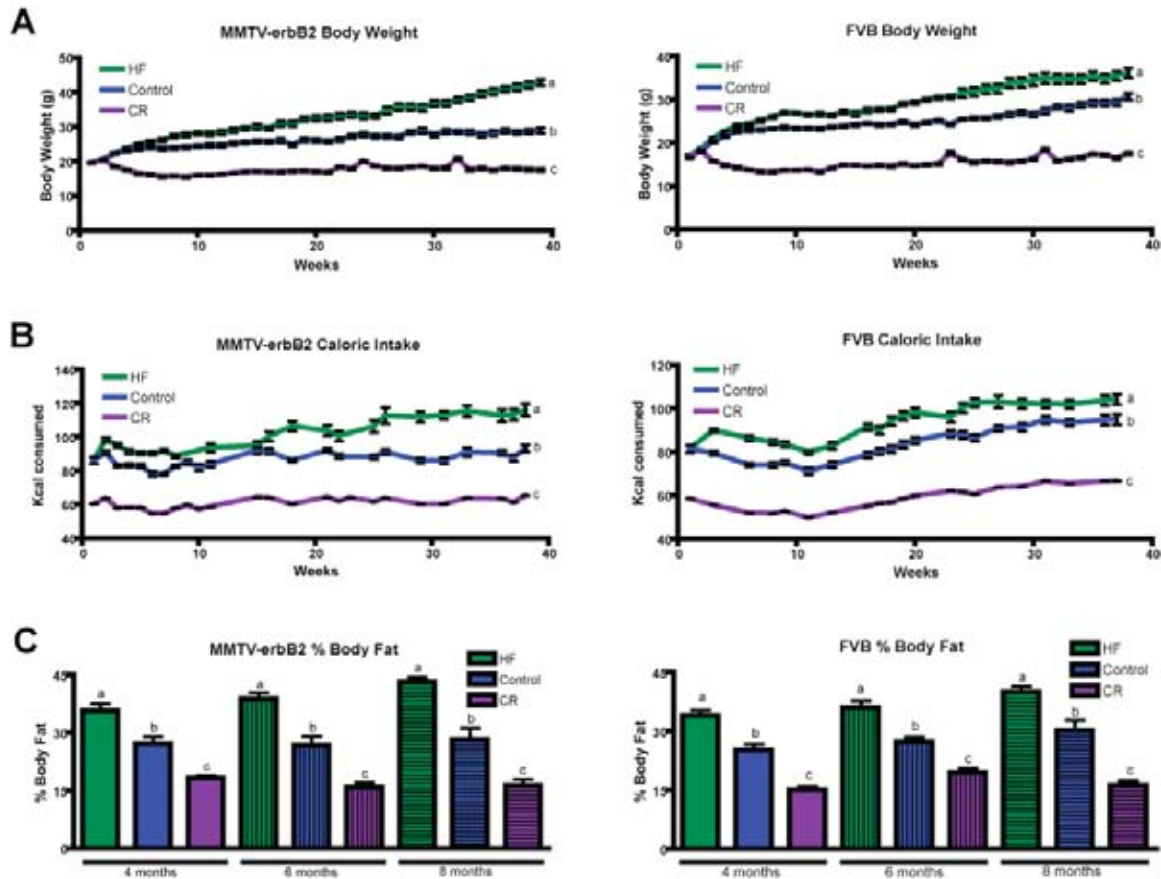
Figure 1. Body Weights, Caloric Intake, and Body Composition of mice. (A) Body weights for MMTV-erbB2 and age-matched non-transgenic (FVB) controls. Body weights were recorded weekly (AVG $\pm$ SEM). CR mice have the lowest body weights and those consuming the HF diet, the highest body weights. (B) Caloric intake graphs: caloric intake was recorded weekly, (AVG $\pm$ SEM). The CR mice consumed the least amount of calories, while the CON and HF mice consumed roughly 30% more per week. (C) % Body fat graphs; body compositions were analyzed at 4, 6, and 8 months (AVG $\pm$ SEM). HF mice in all groups show highest percentage body fat, while the CR mice show the lowest percentage body fat. Different letters represent significant differences between groups ($p<0.0001$).
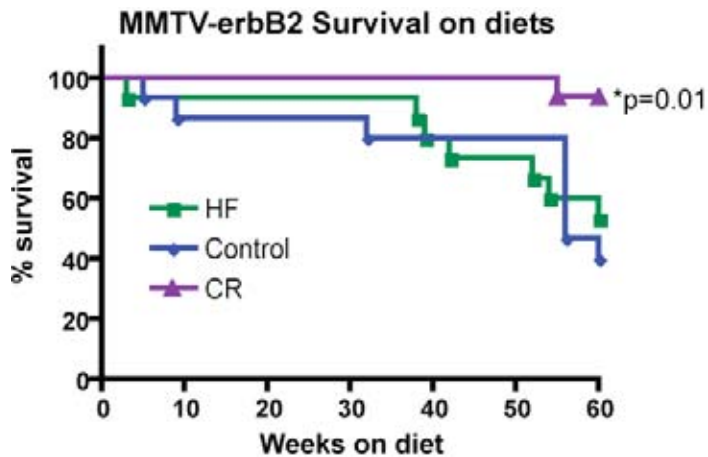
Figure 2. Calorie restriction significantly increases lifespan in MMTV-erbB2 animals. MMTV-erbB2 mice were placed on 3 diets (15/group) for 60 weeks: high fat diet, which induces obesity (HF; 60% kcal from fat), control diet which induces a slightly overweight animal (10% kcal from fat), and 30% calorie restriction (CR; 30% reduction in calories from Control ad-libitum diet). CR significantly increased lifespan after 60 weeks of feeding (p=0.01).
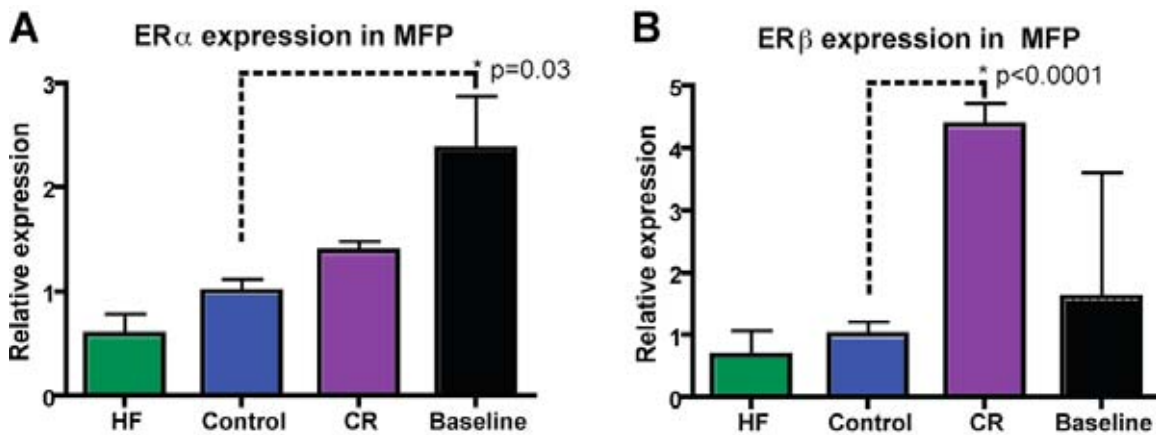
Figure 3. ERα/β mRNA expression in MFP of 8 week-old animals. (A) ERα relative expression in MMTV-erbB2 mice. Baseline animals were 6 weeks/old (chow diet); diet group mice were 8 weeks/old. There was a significant loss of ERα expression in the older diet group mice (Baseline vs. Control; p=0.03). (B) ERβ relative expression at same ages as Panel A. CR significantly increases ERβ expression (CR vs. Control; p<0.0001). Data is AVG $\pm$ SEM.

Statistical Analysis: One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant differences test was used to assess the effects of diet on body weight, caloric intake, and body composition. Unpaired student's t-test was used to assess differences in gene expression, and the Kaplan-Meier test was used to assess differences in survival curves.